

**WS17.1 Biofilm disruption and potentiating antimicrobial effects of a novel alginate oligomer on *Pseudomonas aeruginosa* in a murine lung infection model**

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*P. aeruginosa* growing as a biofilm is the main cause of chronic lung infection in cystic fibrosis (CF) patients. A novel alginate oligomer (OligoG CF-5/20) has been shown to disrupt biofilm and potentiate activity of selected antibiotics. Recent studies have demonstrated a synergistic effect of OligoG with colistin in disrupting biofilms of *P. aeruginosa* NH57388A *in vitro*. The aim of this study was to determine *in vivo* effect of OligoG in a murine biofilm lung infection model. OligoG CF-5/20 is a linear sodium alginate oligomer with an average degree of polymerisation Dp 13 comprising predominantly  $\alpha$ -L-guluronate (AlgiPharma AS, Norway). A clinical isolate of *P. aeruginosa* NH57388A obtained from the lung of a CF patient was used in this model. A biofilm was simulated using NH57388 ( $1 \times 10^8$  cfu/ml) embedded in alginate beads using Pronova LVG (FMC Biopolymer/NovaMatrix, Norway). Balb/cj mice (8 mice/group) were lightly anaesthetized and 0.04 mL of the bead embedded NH57388A administered by intra-tracheal route to the lower left lung of mouse, with or without 5% OligoG (50 mg/mL). A single dose of colistin was administered intraperitoneally 2hr after bacterial challenge. The concentrations of colistin used were 0.4 mg/kg, 1.6 mg/kg and 6.4 mg/kg. Lungs were removed at sacrifice 24hr after bacterial challenge to determine bacterial counts and macrophathology. Results with colistin-OligoG combinations were significantly better than colistin alone with almost three log reduction in lung bacterial burden. Furthermore, a two log reductions in lung bacterial burden was also observed in this model when 5% OligoG was administered alone. Phase IIa clinical trials with OligoG are in progress.

**WS17.2 ALX-109 potentiates the effect of inhaled antibiotics at killing *Pseudomonas aeruginosa* biofilms on human airway cells**

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**Objectives:** Iron has been shown to promote *P. aeruginosa* biofilm formation on airway cells and the iron concentration in the airway surface liquid of CF lung is 400-times higher than in a non-CF lung. Chelating iron may be a promising new therapy to eliminate biofilms on CF airway cells. Here, we investigate whether *P. aeruginosa* biofilms would become more susceptible to the action of inhaled tobramycin (Tb) and inhaled aztreonam in the presence of ALX-109, a new investigational drug containing lactoferrin – an iron-binding glycoprotein – and hypothiocyanite.

**Methods:** Biofilms were grown at the apical surface of polarized human CF airway epithelial cells, using a co-culture model we previously described.

**Conclusion:** ALX-109 and Tb (5  $\mu$ g/ml) together decreased established PAO1 biofilms by 7 log units, an effect significantly larger than Tb alone ( $p < 0.05$ ). The efficacy of ALX-109 and Tb together was also tested on biofilms formed by clinical *P. aeruginosa* strains isolated from the sputum of CF patients. The combination of Tb and ALX-109 was additive for mucoid clinical isolates, reducing biofilms by 2.5 to 3 log units, compared with  $\sim 1$  log unit for each compound alone ( $p < 0.05$ ). At all doses of Tb tested (5–500  $\mu$ g/ml), ALX-109 significantly disrupted biofilms compared with Tb alone. Similarly, the combination of ALX-109 and aztreonam reduced biofilm formation on airway cells by 4 log units, compared with 0 and 3 log units, respectively, for each compound alone.

Inhalation therapy combining tobramycin or aztreonam with ALX-109 may be beneficial to CF patients infected with *P. aeruginosa*. Phase I clinical trials are planned.

**WS17.3 Allicin revisited: Antimicrobial activity against the *Burkholderia cepacia* complex and interaction with a peroxidase target**

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The antimicrobial activity of garlic and other alliums is primarily attributed to allicin [1], an alliin-derived unstable thiosulphinate (allyl 2-propenethiosulfinate) present in crushed garlic bulbs but usually lost in medicinal formulations. In response to the challenges of treating *Burkholderia cepacia* complex (Bcc) infections with existing antimicrobials [2], we sought to identify allicin in aqueous garlic extracts (AGE) and investigate bactericidal activity against a Bcc panel. We used hplc and electrospray ionisation mass spectrometry (LC ESI-MS) to characterise a pure allicin standard and calculate the allicin content of fresh garlic extracts [3,4]. We report the first evidence for the bactericidal activity of both purified allicin and AGE against the Bcc. MICs of AGE for 38 Bcc strains ranged from 0.5 to 3%, MIC90 1%. In agar diffusion assays, AGE was more active against *B. cenocepacia* J2315 than the existing agents ceftazidime, ciprofloxacin and meropenem. The mechanisms involved in the bactericidal action of allicin are poorly understood. Studies on the interaction of allicin and an enzyme target (recombinant BCP peroxiredoxin (Bc BCP Prx) from *Burkholderia cenocepacia* J2315) [5] using Fourier transform ion cyclotron resonance MS (FT-ICR MS) suggest that allicin reacts with the peroxidatic Cys44 residue of the Bc BCP Prx to produce a new mixed disulfide derivative.

**Reference(s)**

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**WS17.4 Anti-pseudomonal bacteriophage cocktail reduces inflammatory responses in the murine lung**

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We hypothesised that bacteriophage cocktails (BPC) may be useful against *Pseudomonas aeruginosa* (Pa) in cystic fibrosis (CF) & tested this in a murine model.

**Methods:** Two Pa strains were tested:

- a. a non-mucoid, clinical CF strain,
- b. Pa01.

Both were sensitive to BPC *in vitro* on standard plaque assays.

Adult BALB/c mice were inoculated intranasally with Pa and BPC (treated, n=21) or buffer (control, n=21). 12 mice were sacrificed 24hrs post-infection and the others at 48hrs. Bronchoalveolar lavage (BAL) was serially diluted for quantitative culture; spleen homogenate was cultured in a non-quantitative fashion. Total and differential cell counts were performed on BAL and inflammatory markers were measured.

**Results:** All treated mice cleared infection at 24 hrs (n=6) compared with none of the controls (n=6) in whom there was a median [range] CFU/ml 1305 [190–4700],  $p < 0.01$ ; inflammatory cell counts at this time point did not differ. At the 48hr time point all mice had cleared infection but there were significantly fewer neutrophils in BAL of treated mice compared with controls (median [range] 174 [112.1–266.8]  $\times 10^4$ /ml vs. 73.2 [35.2–102.1],  $p < 0.01$  for the clinical strain; median [range] 206 [160.1–331.6]  $\times 10^4$ /ml vs. 122.1 [105.4–187.4],  $p < 0.01$  for Pa01). Cytokines including IL-12p70, IL-6, TNF- $\alpha$  and IL-10 were significantly lower in treated mice than controls.

**Conclusions:** BPC co-administration led to

- a. more rapid clearance of Pa and
- b. a reduced inflammatory response in this acute murine model.

Further work is underway to explore the therapeutic potential of bacteriophage in pulmonary Pa infection; preliminary data where BPC is administered 24hrs after initial infection shows promise.